Marine Biology 11, 191—197 (1971)

Toxicity and distribution of Aroclor® 1254 in the pink shrimp *Penaeus duorarum**

D. R. Nimmo, R. R. Blackman, A. J. Wilson, Jr. and J. Forester

Environmental Protection Agency, Gulf Breeze Laboratory; Sabine Island, Gulf Breeze, Florida, USA

Abstract

The polychlorinated biphenyl Aroclor® 1254 was released in an accidental leakage of heat-exchange fluid from an industrial plant, into the Escambia River, near Pensacola, Florida, USA. This material was carried downstream, and is now found in the fauna of Escambia Bay and its contiguous waters, prime nursery areas for fishes and invertebrates such as penaeid shrimp. The significance of pollution by this chemical was assessed by establishing toxicity levels, determining routes of entry, and investigating its movement and distribution in various tissues of shrimp under controlled conditions in the laboratory. Aroclor 1254 added to the water was toxic to the juvenile pink shrimp *Penaeus duorarum* at a concentration of 1.0 part per billion within 15 days, but was less toxic to adult pink shrimp. Shrimp obtained the contaminant from water and food and concentrated it to 510.0 parts per million in the hepatopancreas. Aroclor 1254 residue data from shrimp collected in the estuary are included in the study.

Introduction

Since 1966, pesticide toxicologists and ecologists have become increasingly aware of polychlorinated biphenyls (PCBs). First discovered in fishes, feathers, and human hair (Jensen, 1966), residues have since been found in many organisms from diverse areas of the world. Structurally, PCBs resemble chlorinated hydrocarbon pesticides such as DDT, and are widely used in formulating plastics, resins for rubber-based lacquers, varnishes, paints, lubricants, heat-transfer fluids and electrical insulators. PCBs are relatively

® Registered trademark, Monsanto Company, St. Louis, Missouri. Mention of commercial products does not constitute endorsement by the Environmental Protection Agency.

* Contribution No. 128, Gulf Breeze Laboratory.

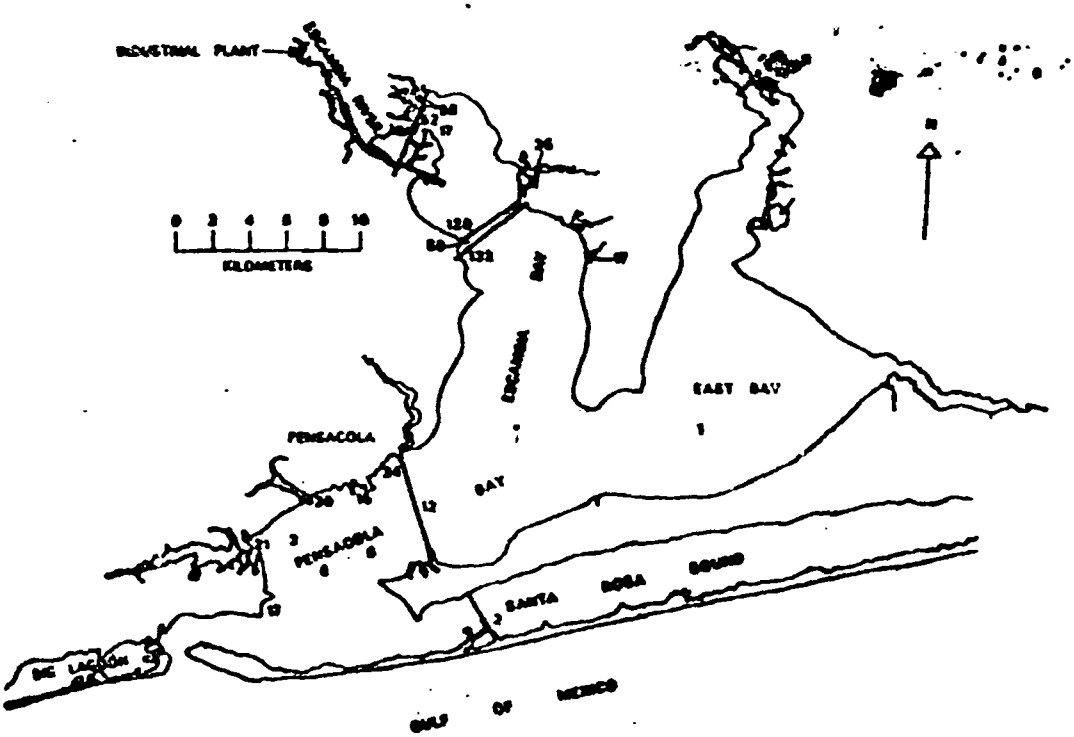

Fig. 1. Residues of Aroclor 1254 (in ppm) found in shrimp hepatopancreases from Escambia Bay and contiguous waters during 1969/1970. Each sample listed represents composite tissues of at least 5 individuals

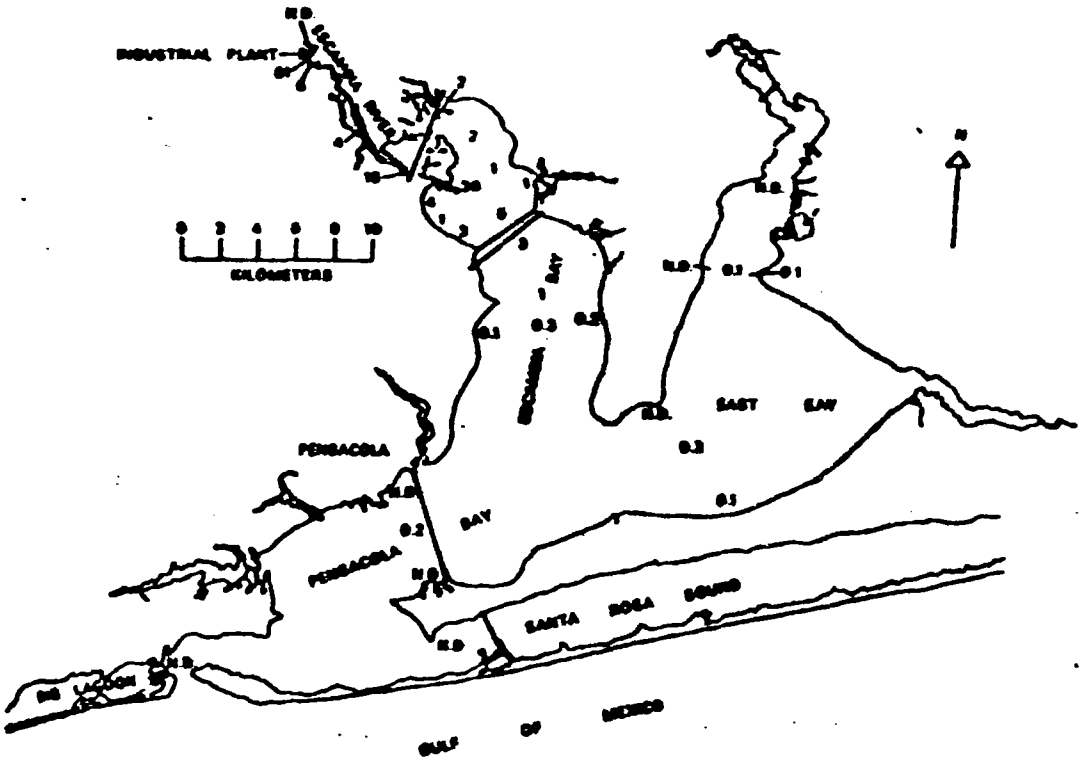

Fig. 2. Residues of Aroclor 1254 (in ppm) found in sediments from Escambia Bay and contiguous waters during 1969/1970. N.D.: less than 0.03 ppm

insoluble in water, but soluble in lipid and lipid solvents. In addition to their thermal stability they are also resistant to acid and base, and therefore, persist in the environment.

PCBs are toxic to trout and blue gill (spp. not given) — GUSTAFSON, 1970; shrimp (*Penaeus duorarum*) and oysters (*Crassostrea virginica*) — DUKE et al., 1970; and a fish (*Lagodon rhomboides*) — HANSEN et al., 1971. Also, a PCB used as a binder in epoxy paint was toxic to chickens (GUSTAFSON, 1970). Abnormally thin-shelled eggs of birds in Great Britain and North America were associated with residues of chlorinated hydrocarbons including the PCBs (RISEBROUGH et al., 1968). In 1969, a PCB (Aroclor 1254) was discovered as a contaminant in water, sediment and fauna of Escambia Bay, Florida (DUKE et al., 1970). One source of this material was traced to an accidental leak in a heat-exchange system of an industrial plant located several kilometers upstream in Escambia River. It is now present in estuarine organisms, including shrimp captured from Escambia Bay and contiguous waters (Fig. 1). Sediments from the river and upper bay appear to be a reservoir for the compound (Fig. 2). In earlier experiments, shrimp exposed to these sediments for 30 days accumulated the chemical (NIMMO et al., 1971). In this paper we report toxicity of Aroclor 1254 in water, rates of accumulation from food and water, and distribution of this PCB in the organs of the pink shrimp *Penaeus duorarum*.

Materials and methods

Shrimp for laboratory studies were obtained from two sources. Juvenile (2.5 to 3.8 cm) pink shrimp (*Penaeus duorarum*) were collected with a small net from Santa Rosa Sound at Pensacola Beach, Florida, in June through September. Adult pink shrimp from Tampa, Florida, were purchased from a live-bait dealer. Background concentrations of chlorinated hydrocarbon compounds in the hepatopancreases of all shrimp never exceeded 0.6 parts per million (ppm); whole-body residues were less than 0.01 ppm.

All shrimp were acclimated in flowing sea water for several days in the laboratory. Juveniles fed on detritus carried in by the flowing unfiltered sea water and adults were fed mullet (*Mugil cephalus*) muscle containing less than 0.03 ppm organochlorine compounds each day. Beach sand with no detectable organochlorine compounds was provided as a substrate for the shrimp.

Shrimp were exposed to Aroclor 1254 (hereafter called Aroclor) in flowing-water systems. The Aroclor was dissolved in polyethylene glycol 200, infused into the flowing water with syringe pumps, then mixed by

a series of baffles as it flowed into aquaria. The volume of the aquaria varied from 18 to 160 l commensurate with the numbers and sizes of test animals and flow-rate of water. Animal to volume ratio was 1 animal per 2 l water. Concentrations of Aroclor were routinely measured by gas chromatography. No attempt was made to control salinities which ranged from 25 to 32‰, but electric aquarium heaters were used to maintain water temperatures between 20° and 30 °C.

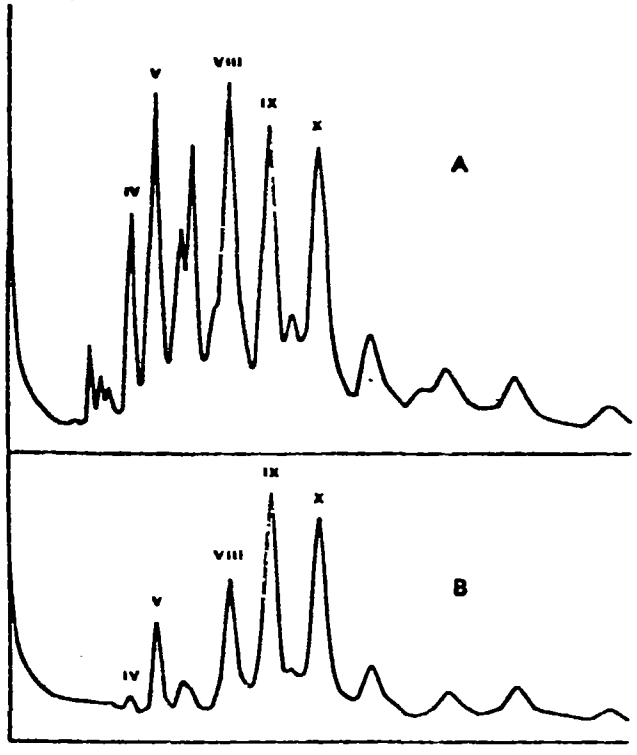

Fig. 3. Aroclor chromatograms. (A) Aroclor 1254 standard, (B) Aroclor 1254, recovered from *Penaeus duorarum* hepatopancreas after shrimps had been fed fish containing material (see Table 4). Concentrations of DDT and its metabolites were negligible, therefore, no attempt was made to separate them from Aroclor isomers. Gas flow 25 ml/min, nitrogen; injection temperature 210 °C, oven temperature 190 °C, detector temperature 210 °C; H^3 electron capture detector; 152.4 × 0.317 cm glass-column packed with 2% OV-1 on 100/120 Gas Chrom Q

Concentrations of Aroclor in shrimp were determined from pooled samples by gas chromatography. When a group of 10 shrimp was exposed to a constant concentration of Aroclor in flowing-water, individual residues differed by a factor of 10. Consequently, laboratory analyses are from composite samples of at least 10 individuals, unless stated otherwise. All analyses on *Penaeus duorarum* from Escambia Bay and contiguous waters were on composite samples of at least 5 individuals.

Depending on type and weight of the samples, four methods of preparation were employed. (1) Samples of shrimp or food items larger than 1 g were mixed with anhydrous sodium sulfate in a blender and extracted for 4 h with petroleum ether in a Soxhlet apparatus. Extracts were concentrated and eluted from a Florisil column with 6% ethyl ether in petroleum ether. (2) Samples less than 1 g were analyzed by a modification of the micro-method of Enos (private communication). Samples were weighed in Duall[1] tissue grinders and extracted with three 2.0 ml portions of acetonitrile. The acetonitrile extracts were combined and diluted with 6 ml of 2% Na_2SO_4 in distilled water, then agitated and extracted with three 2.0 ml portions of hexane. These extracts were combined and concentrated to about 0.5 ml, then transferred to a Size "B" Chromaflex[1] column containing 1.5 g of Florisil topped with 1.5 g of anhydrous sodium sulfate. The residue was eluted from the column with 20.0 ml of 1% ethyl ether in hexane. (3) Water samples were extracted with petroleum ether, then the extracts were dried with anhydrous sodium sulfate and reduced to an appropriate volume. (4) Sediments were analyzed by the method of Nimmo et al. (1971).

All eluates were adjusted to an appropriate volume for analysis by electron-capture gas chromatographs equipped with OV-1 columns. Quantitation of Aroclor 1254, a multiple-peaked compound, was made by averaging the heights of 5 major peaks which had retention times relative to aldrin of 1.31 (*IV*), 1.55 (*V*), 2.32 (*VIII*), 2.74 (*IX*) and 3.27 (*X*) (Fig. 3). Interference from DDT was negligible due to the relatively high residues of Aroclor 1254 in most samples. Laboratory tests indicated recovery rates above 80%, but data in this report do not include a correction factor for recovery. The presence of Aroclor 1254 in shrimp and sediments was verified by mass spectroscopy at the Environmental Protection Agency Laboratory, Athens, Georgia.

Results

Acute and chronic biosassays

Acute toxicity tests at this laboratory showed that Aroclor was about one tenth as toxic to juvenile *Penaeus duorarum* as DDT (Table 1). For example, 10.0 parts per billion (ppb) DDT in the water killed 100% of a population of shrimp in 96 h, whereas 100.0 ppb in the water was necessary to obtain the same results with Aroclor.

In chronic flowing-water bioassays, Aroclor at 0.04 ppb killed 51% of the juvenile shrimp (2.5 to 3.8 cm) within 15 days (Table 2). Juvenile shrimp were more sensitive to Aroclor than adults. Exposure to 3.5 ppb for 35 days resulted in a mortality of 50% in a group of adult shrimp (9.5 to 12.5 cm). The data

[1] Kontes Glass Co., Vineland, N. J., USA.

show the need for challenging several stages in the life cycle of shrimp with chemicals such as Aroclor.

We have observed that symptoms of Aroclor poisoning in shrimp are different from those of most organochlorine insecticides. Pink shrimp which we exposed to 0.15 ppb or more DDT showed nervous impairments such as tremors, loss of equilibrium and, finally, paralysis as defined by cessation of locomotor movements. In tests with Aroclor, regardless of its concentration, shrimp showed delayed mortality and died at a rate of one or two per day with no apparent prior symptoms of poisoning. Like others (Duke, et al., 1970; Wildish, 1970), we suggest that crustaceans may be more susceptible to the chemical during molting.

Table 1. *Penaeus duorarum. Comparison of toxicities of p, p' – DDT and Aroclor 1254 to shrimp in flowing-water tests. Mean temperature and salinity of sea water in DDT experiment were 24 °C and 28 ‰, respectively; for Aroclor 1254, 19 °C and 31 ‰*

DDT[a]			Aroclor 1254[b]		
Test concentration (ppb)	Mortality 48 h (%)	96 h (%)	Test concentration (ppb)	Mortality 48 h (%)	96 h (%)
10.0	100	100	100.0	80	100
1.0	30	80	10.0	0	0
0.5	0	40	1.0	0	0
0.1	10	20	Control	0	0
Control	0	0			

[a] Personal communication, J. I. Lowe, Environmental Protection Agency, Gulf Breeze, Florida 32561, USA.
[b] Duke et al., 1970.

Accumulation and transfer of Aroclor in the

The uptake of Aroclor from water by adult *Penaeus duorarum* (3.8 to 7.6 cm) and the transfer to the hepatopancreas, whole body and abdominal muscle was measured (Fig. 4). Accumulation was linear with time in the hepatopancreas ($r = 0.97$) and whole body ($r = 0.90$), but a plateau was reached

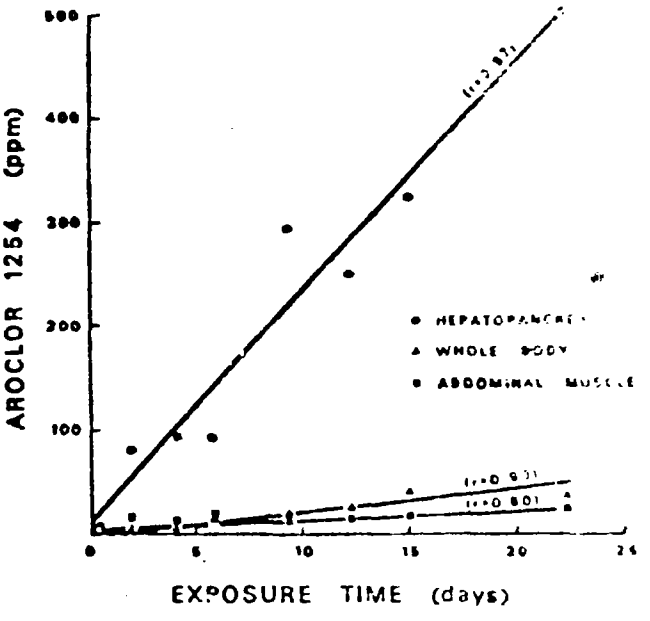

Fig. 4. *Penaeus duorarum*. Rates of absorption of Aroclor 1254, in various tissues of shrimp exposed to 2.5 ppb of the chemical in flowing water. Unexposed shrimp showed no detectable residue. r = correlation coefficient

Table 2. *Results of chronic bioassays with Aroclor 1254 and the pink shrimp Penaeus duorarum in flowing water*

Shrimp rostrum-telson length (cm)	Concentration[a] (ppb)	Average salinity (‰)	Average temperature (°C)	No. of test individuals	Replicates	Days exposed	Average mortality (%)	Level of significance
2.5 – 3.8	Control	32	29	65	5	15	12	
2.5 – 3.8	0.57	32	29	20	2	15	30	0.10[b]
2.5 – 3.8	0.94	32	29	45	3	15	51	0.005[b]
2.5 – 3.8	9.4	32	29	20	2	15	90	0.001[b]
2.5 – 3.8	19.0	32	29	20	2	15	100	0.001[b]
4.2 – 7.2	Control	29	28	25	1	32	4	—
4.2 – 7.2	2.4	29	28	20	1	17	65	0.001[c]
4.2 – 7.2	3.1	29	28	25	1	32	80	0.001[c]
6.6 – 9.0	Control	29	20	43	1	53	26	—
6.6 – 9.0	4.3	29	20	46	1	53	83	0.001[c]
7.6 – 8.5	Control	31	29	60	1	18	9	—
7.6 – 8.5	4.0	31	29	60	1	18	41	0.001[c]
9.5 – 12.5	Control	28	20	50	1	35	8	—
9.5 – 12.5	3.5	28	20	50	1	35	50	0.001[c]

[a] Average of at least three determinations.
[b] Student's *t*-test.
[c] Chi-square.

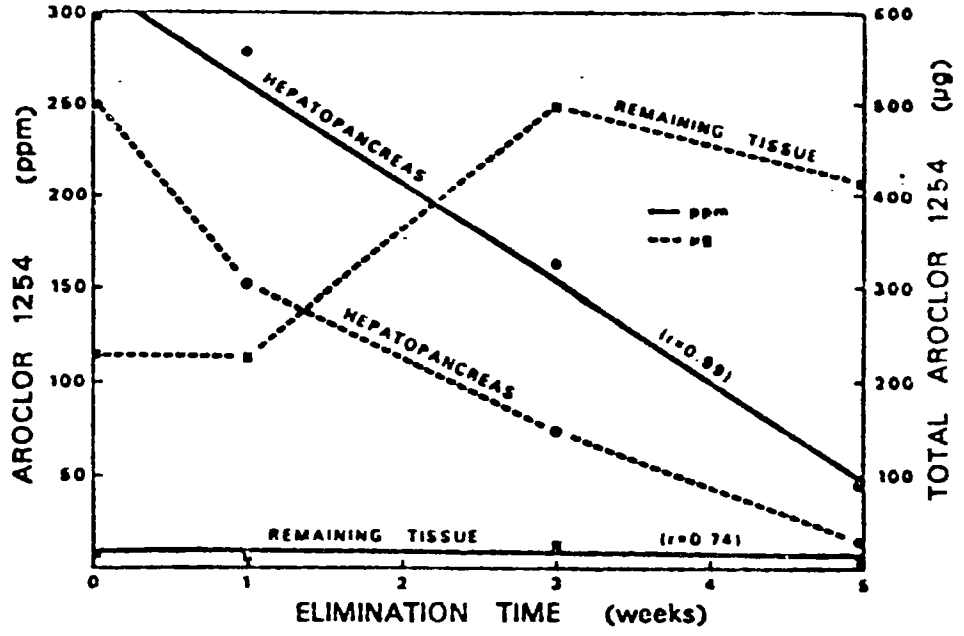

Fig. 5. *Penaeus duorarum*. Rates of elimination from hepatopancreas and subsequent increase in remaining tissue. Shrimp were exposed to 7.5 ppb Aroclor 1254 in flowing water for 16 days, then placed in Aroclor-free environment. Composite tissue samples from 8 individuals were analyzed for each determination. Unexposed shrimp showed no detectable residues
r = correlation coefficient

in the muscle within 2 days, with little increase thereafter. Residues in the hepatopancreas reached 510.0 ppm after 22 days and represented a 2.04×10^5 increase over the 2.5 ppb Aroclor in the test water. During this experiment, 50% of the exposed and 7% of the unexposed shrimp died.

In a subsequent experiment, most of the Aroclor was lost from the hepatopancreas and transferred to other tissues (Fig. 5). Adult shrimp (9.5 to 11.5 cm) were first exposed to 7.5 ppb Aroclor in the water for 16 days, then placed in an Aroclor-free environment for 5 weeks. Total weight (µg) in the hepatopancreas decreased by 80% in 5 weeks, but that in the remaining tissues almost doubled. Whole-body loss was from 731 to 466 µg or about 60% in 5 weeks.

A slightly different picture exists concerning the loss of Aroclor from the tissues if we express the amount in parts per million (Fig. 5). Aroclor (in ppm) showed little change in tissues other than the hepatopancreas during post exposure. In contrast, the rate of elimination from the hepatopancreas was constant and linear with time ($r = 0.99$), the biological halflife in this organ being 17 days. Aroclor is more persistent in the other tissues of shrimp than DDT, the insecticide being completely eliminated in 3 weeks (NIMMO et al., 1970). While the *Penaeus duorarum* were held in the Aroclor-free environment, 23% of the exposed shrimp died, with no loss of the unexposed.

Accumulation in body organs

Residues found in laboratory experiments are compared with those in natural populations of shrimps in Escambia and Pensacola Bays in Table 3. In all tests, the shrimp incorporated the chemical. The proportion of Aroclor in tissues of shrimp which were exposed to 0.2 ppb in the water for 50 days was nearest to that found in shrimps captured alive from the bays.

The distribution of Aroclor in the tissues of shrimp is much the same as DDT, maximum amounts occurring in the hepatopancreas and least in abdominal muscle or exoskeleton (NIMMO et al., 1970). Generally, the distribution of Aroclor in the tissues of *Penaeus duorarum* corresponded to the amount of lipid in tissues.

We believe water and food are sources of Aroclor to shrimp, but we do not know which contributes more. FARFANTE (1969) summarized earlier work on the feeding habits of shrimp and reported that the three commercially-important penacid shrimps, pink *Penaeus duorarum*, white *P. setiferus*, and brown *P. aztecus*, are omnivorous. Some of the contents found in digestive tracts by other investigators include inorganic debris, detritus, and a variety of algae, including diatoms. Aroclor attached to detrital material in aquaria or in field substrates was probably ingested by the shrimp.

Table 3. *Distribution of Aroclor 1254 in tissues of exposed Penaeus duorarum which had accumulated chemical from water and food and in penacid shrimps from natural populations in the Pensacola estuary, Florida, USA*

Methods of exposure	Hepato-pancreas ppm	Ventral nerve	Digestive tract	Heart	Gills	Exo-skeleton	Abdominal muscle
Water							
Pink shrimp exposed to 3.5 ppb Aroclor in water for 35 days	168	120	32	77	59	14	15
Pink shrimp exposed to 0.2 ppb Aroclor in water for 50 days	30	3.3	3.2	2.8	1.4	0.8	0.7
Food							
Pink shrimp fed spot[a] (43.0 ppm whole body) for 16 days	146	30	15	25	38	6.5	57
Pink shrimp fed spot (field-captured, 0.2 ppm whole body) for 16 days	0.6	0.5	<0.1	0.3	0.3	0.2	<0.1
Pink shrimp fed croaker[b] (0.68 ppm in muscle) for 50 days	5.8	2.0	1.0	1.3	0.9	0.6	0.6
Natural populations							
Pink shrimp captured 19. I. 1970[c]	15	9.4	1.1	—	1.4	0.6	0.9
Pink shrimp captured 3. IV. 1970[c]	4.6	0.6	0.5	0.5	0.3	0.2	0.1
White shrimp captured 28. VIII. 1970[d]	17	4.0	1.5	1.0	0.8	0.8	1.3
Brown shrimp captured 28. VIII. 1970[d]	98	14	9.7	4.2	3.1	2.2	0.9

[a] The spot *Leiostamus xanthurus* were previously exposed to 5.0 ppb Aroclor 1254 in the water for 12 days.
[b] Atlantic croaker *Micropogon undulatus* were captured in Escambia Bay, Florida.
[c] Pensacola Bay.
[d] Escambia Bay.

Table 4. *Percentages[a] of 5 peaks in Aroclor 1254 recovered from water, fish and Penaeus duorarum*

Item	Peak (%)				
	IV	*V*	*VIII*	*IX*	*X*
Standard	10.0	18.5	25.0	22.2	20.0
Water (30‰ S)	8.2	15.6	30.0	22.8	23.1
Fish muscle[b]	5.1	10.8	27.4	27.9	28.4
Shrimp hepatopancreas[c]	2.8	10.9	23.9	31.1	31.1

[a] $\frac{\text{peak height}}{\text{sum of 5 peaks}} \times 100$.
[b] The Atlantic croaker *Micropogon undulatus* were captured from Escambia Bay and contained 0.68 ppm Aroclor 1254.
[c] The shrimp *Penaeus duorarum* were fed Atlantic croaker muscle.

We investigated differences in the proportion of Aroclor peaks with respect to the standard; the change is greatest in the hepatopancreas of shrimp (Fig. 3; Table 4). Heights of 5 peaks used for quantitation of the chemical show the greatest reduction in peaks *IV* and *V*, with some increase in *IX* and *X*. We do not know whether this reflects actual alterations in the molecules or differential solubilities in the various systems.

Results of field studies

Distribution of shrimp in the estuary in relation to salinity is a factor which regulates the amount of Aroclor in the body (Fig. 1). The brown shrimp *Penaeus aztecus* from upper Escambia Bay had the highest residues (132.0 ppm in the hepatopancreas). The white shrimp *P. setiferus* from the mouths of small streams emptying into Escambia Bay had a maximum residue of 59.0 ppm. The highest residue in the pink shrimp *P. duorarum* captured in Pensacola Bay was 15.0 ppm. *P. setiferus* is most abundant in low salinity waters of less than 10‰ and *P. aztecus* occurs mostly in waters of 10‰ or more, the abundance of *P. duorarum* is not as dependent on salinity (Farfante, 1969). Although this distribution may vary with locale, types of substrate and seasonal temperatures, shrimp with the highest residues in this study were those captured in the lower salinities. Because higher concentrations of Aroclor occur in the sediments of upper Escambia Bay (Fig. 2), it is possible that burrowing activities of brown shrimp in these sediments could have caused additional absorption of leached chemical through the gills (Nimmo et al., 1971) as well as ingestion of contaminated food.

Discussion and conclusions

In our investigations, there has been no evidence that Aroclor in the water, sediments, or biota in Escambia Bay was toxic to shrimp. We found no dead or dying shrimp in areas of "fish kills" which occurred frequently during summer months of the past two years. Aroclor in water samples collected 48 cm above the sediments in the upper Bay was below that considered toxic to shrimp, but it was detectable (0.06 ppb in unfiltered water). It probably leached from the sediments or was attached to suspended particulate matter. Adult shrimp in live cages placed directly on sediments in upper Escambia Bay for 3 weeks did not die nor did the "controls" held 48 cm above in an uncontaminated substratum. Shrimp on lower sediments accumulated almost 3 times more Aroclor (6.7 ppm in the hepatopancreas) than did controls.

Nevertheless, our laboratory investigations show that Aroclor 1254 in solution is toxic in the 1 ppb range to shrimp. Therefore, the occurrence of this chemical in the water of Escambia Bay or in other estuarine areas is reason for concern. We also believe the residues found in shrimp from the Escambia Bay are high enough to be of importance, although we have not found a correlation between residues and mortality. If postlarval or juvenile shrimp were exposed directly to the sediments in upper Escambia Bay for a period of weeks, a threat could exist because of availability of PCB-laden detritus and also the leaching of the chemical at the water-substrate interface. We are now investigating this possibility.

Deaths of shrimp due to this or any other contaminant in natural environments would be difficult to observe. Except as larvae, shrimp are primarily benthic, secretive animals, and hide by burrowing in the sediment. If they are active at night, they usually remain below the substrate by day. Here, they may obtain higher concentrations of Aroclor than when swimming, and if they die, dead shrimp do not surface after dying as do fish, rather they decompose rapidly or are quickly eaten by predators.

Aroclor occurs in the tissues of shrimp which were captured several kilometers from the original sources of the material in Escambia Bay (Fig. 1). This suggests the potential of this material to be dispersed through the ecosystem, and it might be available to man through his seafood. We believe this contamination should serve as a warning to increase monitoring of the environment for Aroclor and related industrial materials.

Summary

1. A concentration of 1.0 part per billion of Aroclor in the water kills the juvenile pink shrimp *Penaeus duorarum* within 15 days in the laboratory. Adult shrimp are not as susceptible, but higher concentrations of 2.4 to 4.3 ppb kill within 17 to 53 days — depending on the maturity of test individuals.

2. In laboratory tests in which Aroclor was added to the water, *P. duorarum* absorbed the material and concentrated it in the hepatopancreas. Subsequently, it was transferred to other tissues, then lost. It was more persistent in the tissues of shrimp than the chemically-related pesticide, DDT.

3. Aroclor was absorbed from the water and its subsequent distribution in tissues was similar to that found in wild shrimp from contaminated areas. In the laboratory, shrimp also obtained the Aroclor from food, and we conclude that both sources are available to shrimp in the Pensacola estuary.

4. Concentrations of Aroclor in *P. duorarum* from the estuary appear to reflect distribution patterns in shrimp. Aroclor in shrimp captured at considerable distances (up to 24 km) from the original source demonstrate the potential for dispersion and concentration of this material in an ecosystem.

Literature cited

Duke, T. W., J. I. Lowe and A. J. Wilson, Jr.: A polychlorinated biphenyl (Aroclor 1254) in the water, sediment, and biota of Escambia Bay, Florida. Bull. Envir. Contam. Toxicol. 5, 171—180 (1970).

Farfante, I. P.: Western Atlantic shrimps of the genus *Penaeus*. Fishery Bull. Fish Wildl. Serv. U.S. 67, 461—491 (1969).

Gustafson, C. G.: PCB's-prevalent and persistent. Envir. Sci. Technol. 4, 814—819 (1970).

Hansen, D. J., P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr. and P. D. Wilson: Chronic toxicity, uptake, and retention of Aroclor® 1254 in two estuarine fishes. Bull. Envir. Contam. Toxicol. 6, 113—119 (1971).

Jensen, S.: Report of a new chemical hazard. New Sci. 32, 612 (1966).

Nimmo, D. R., A. J. Wilson, Jr. and R. R. Blackman: Localization of DDT in the body organs of pink and white shrimp. Bull. Envir. Contam. Toxicol. 5, 333—341 (1970).

—, P. D. Wilson, R. R. Blackman and A. J. Wilson, Jr.: Polychlorinated biphenyl absorbed from sediments by fiddler crabs and pink shrimp. Nature, Lond. 231, 50—52 (1971).

Risebrough, R. W., P. Rieche, D. B. Peakall, S. G. Herman and M. N. Kirven: Polychlorinated biphenyls in global ecosystem. Nature, Lond. 220, 1098—1102 (1968).

Wildish, D. J.: The toxicity of polychlorinated biphenyls (PCB) in sea water to *Gammarus oceanicus*. Bull. Envir. Contam. Toxicol. 5, 202—204 (1970).

First author's address: Dr. D. R. Nimmo
Environmental Protection Agency
Gulf Breeze Laboratory
Sabine Island
Gulf Breeze, Florida 32561
USA

Date of final manuscript acceptance: June 23, 1971. Communicated by J. Bunt, Miami